

SEROPREVALENCE OF HIV, HTLV-I/II AND OTHER PERINATALLY-TRANSMITTED PATHOGENS IN SALVADOR, BAHIA.

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SUMMARY

Generation of epidemiological data on perinatally-transmitted infections is a fundamental tool for the formulation of health policies. In Brazil, this information is scarce, particularly in Northeast, the poorest region of the country.

In order to gain some insights of the problem we studied the seroprevalence of some perinatally-transmitted infections in 1,024 low income pregnant women in Salvador, Bahia.

The prevalences were as follow: HIV-1 (0.10%), HTLV-I/II (0.88%), *T.cruzi* (2.34%), *T.pallidum* (3.91%), rubella virus (77.44%), *T.gondii* IgM (2.87%) and IgG (69.34%), HBs Ag (0.6%) and anti-HBs (7.62%). Rubella virus and *T.gondii* IgG antibodies were present in more than two thirds of pregnant women but antibodies against other pathogens were present at much lower rates. We found that the prevalence of HTLV-I/II was nine times higher than that found for HIV-1. In some cases such as *T.cruzi* and hepatitis B infection there was a decrease in the prevalence over the years. On the other hand, there was an increase in the seroprevalence of *T.gondii* infection.

Our data strongly recommend mandatory screening tests for HTLV-I/II, *T.gondii* (IgM), *T.pallidum* and rubella virus in prenatal routine for pregnant women in Salvador. Screening test for *T.cruzi*, hepatitis and HIV-1 is recommended whenever risk factors associated with these infections are suspected. However in areas with high prevalence for these infections, the mandatory screening test in prenatal care should be considered.

KEYWORDS: Pregnant women; Perinatally-transmitted pathogens; HIV; HTLV-I/II; Seroprevalence

INTRODUCTION

Perinatally-transmitted pathogens constitute serious public health problems because they frequently cause clinical disease in the infected mothers and their infants. Pathogens often associated with mother/child perinatally-transmitted infections include: *Toxoplasma gondii*, *Treponema pallidum*, *Trypanosoma*

cruzi, rubella virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV) and human T-cell lymphotropic virus type I (HTLV-I). Once acquired during the pregnancy, some of these pathogens can induce devastating effects on the fetus, causing an array of pathological alterations including abortions,

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still births, early infant deaths, and congenital abnormalities.^{11,15,31,39}

In Brazil, epidemiological information on perinatally-transmitted infections is scarce, particularly in the Northeast, the poorest region of the country.

In order to gain some insights of the problem in this part of Brazil, we have studied the seroprevalence of the above mentioned pathogens, in low income pregnant women living in Salvador, Bahia. This city displays several ethnic and sociodemographic characteristics of large African or Haitian cities² and for this reason, fear of HIV spreading by heterosexual and perinatal transmission exists^{30,34}. We also tried to correlate seroprevalence with sociodemographic variables.

MATERIAL AND METHODS

Study design and population. The city of Salvador is the capital of the State of Bahia, which is located at the Northeastern region of Brazil. The city's population is approximately 2,500,000 inhabitants, mostly of African and Portuguese descent².

Pregnant women referred to the Bahia state public health laboratory for prenatal test were the participants of this study. A total of 1,024 women, all living in Salvador and with an annual family income less than US\$2,400.00 were enrolled between November 1990 and June 1991. At the time of blood samples collection, sociodemographic and clinical data were obtained by questionnaires. All women agreed to participate anonymously in this study. The questionnaire was designed to collect the following data: age, place of birth, level of education, history of spontaneous abortions, number of lifetime sexual partners, use of intravenous drug and blood transfusion. After blood processing, sera were frozen and stored at -20°C until use.

Serological assays. Sera were screened for: a) Human Immunodeficiency Virus type 1 and 2 (HIV 1/HIV 2) antibodies by ELISA (Innogenetics, Belgium) and repeat reactive samples were confirmed by Western blot (Dupont, USA). Western blot was considered positive when antibodies against env (gp 160, 120, and/or 41) and gag proteins (p24) were present; b) Human T lymphotropic virus I/II (HTLV-I/II) antibodies were screened by ELISA (Abbott, USA) and repeat reactive samples confirmed by Western Blot (Cambridge Biotech Corporation, USA). Western blot patterns were interpreted according to the manufacturer's instructions. Briefly, specimens were considered positive when at least the p24 glycoprotein and the gp46 protein or the recombinant polypeptide p21e were present. Sera with no reactivity to HTLV-I/II specific proteins were considered negative and sera with different profiles were indeterminate. Western Blot positive samples were submitted for serological discrimination between HTLV-I and HTLV-II by ELISA. This assay is based on serum se-

lected reactivity to HTLV-I or HTLV-II specific synthetic peptides (Select HTLV, Coulter Corporation, Hialeah, FL, USA); c) *T. gondii* IgG and IgM antibodies were detected by ELISA (Hoechst/Behring, Germany); d) Evidence for the presence of *T. pallidum* infection was initially evaluated by serum reactivity to non-treponemal cardiolipin antigen (VDRL). VDRL positive samples were further tested by *T. pallidum* indirect immunofluorescence (FTA, Hoechst/Behring, Germany). Samples reactive to both VDRL and FTA-ABS were considered positive for the purpose of this study; e) Rubella virus antibodies were detected by hemagglutination inhibition assay (HIA, Bio-Manguinhos, FIOCRUZ, Brazil); f) Hepatitis B (HBs) surface antigens and antibodies were detected by ELISA developed at Bio-Manguinhos, FIOCRUZ, Brazil; and g) *T. cruzi* antibodies were screened by passive hemagglutination and positive samples confirmed by indirect immunofluorescence (Bio-Manguinhos, FIOCRUZ, Brazil).

HBV, rubella virus and *T. gondii* were tested by only one assay. Samples were interpreted as positive if at least 2 out of 3 tests were positive. All assays were performed according to the manufacturer's instructions.

Statistical analysis. Data were analysed by using Chi Square test with Yates's correction and by Fisher exact test. Statistical significant level (p value) and confidence interval (CI) was set to 0.05 and 95% respectively.

RESULTS

The overall seroprevalence for HTLV-I, HIV-1, *T. pallidum*, *T. cruzi*, rubella virus, HBV and *T. gondii* is shown in Table 1. Except for HIV-1, all pathogens had seroprevalence values within a confidence interval that excludes the null value. Rubella virus and *T. gondii* IgG antibodies were present in more than two thirds of preg-

TABLE 1
Seroprevalence of perinatally-transmitted pathogens in pregnant women in Salvador, Bahia

Pathogens	Test		n	Preval. ^c	95% - CI ^d
	1st ^a	2nd ^b			
HIV - 1	ELISA	WB ^c	1024	0.10	0.09 - 0.29
HTLV - I/II	ELISA	WB	1024	0.88	0.31 - 1.45
<i>T. cruzi</i>	PHA ^f	IFA ^g	1024	2.34	1.42 - 3.27
<i>T. pallidum</i>	VDRL	IFA	1024	3.91	2.72 - 5.09
HBV	ELISA	-	1024	7.62	5.99 - 9.24
Rubella virus	HIA ^h	-	1024	77.44	74.88 - 80.00
<i>T. gondii</i>	ELISA	-	-	-	-
	IgM	-	698	2.87	1.63 - 4.10
	IgG	-	698	69.34	65.92 - 72.76

^aScreening test, ^bConfirmatory test, ^cPreval. = prevalence; ^dCI = confidence interval, ^eWestern Blot, ^fPassive hemagglutination assay, ^gImmunofluorescence, ^hHemagglutination inhibition assay.

nant women but antibodies against other pathogens were present at much lower rates.

One woman out of 1,024 was repeatedly reactive by ELISA and confirmed positive by Western blot for HIV-1. She was 24 years old, illiterate, born in Salvador and had a history of abortion. She denied use of intravenous drugs and blood transfusion.

Fourteen women were repeatedly reactive for HTLV-I/II antibodies by ELISA. These sera were then confirmed by Western blot and nine were positive, one was indeterminate and the remaining four were negative. Six out of eight positive samples were discriminated by synthetic peptide ELISA as HTLV-I. Two samples were negative for both HTLV-I and HTLV-II peptides.

Socio-economic and demographic values shown to be associated with each infection are presented in Tables 1,2,3,4 and 5. HTLV I/II positive pregnant women were more likely to be born in Salvador (OR = 9.69, 95% C.I. = 1.29-430.75) and they had more than two lifetime sexual partners (OR = 4.52, 95% C.I. = 1.04-20.30) than reference groups (Table 2). Other variables such as age, level of education and spontaneous abortions were not statistically associated with HTLV. None of the HTLV-I/II positive women had history of blood transfusion or intravenous drug use.

TABLE 2

Factors associated with HTLV - I/II positivity among low income pregnant women in Salvador

Variables	No. of women		OR ^a	95% - CI ^b
	Pos./total	%		
Place of Birth				
Salvador	8/467	1.71	9.69	1.29 - 430.75
Outside Salvador	1/557	0.18	1.00	-
No. of sex partners				
1 - 2	4/499	0.80	1.00	-
>2	5/142	3.52	4.52	1.04 - 20.30

^aOdds Ratio, ^bConfidence Interval

T. cruzi antibodies were detected in 24 samples (2.34%, Table 1). Illiteracy was the strongest risk factor for *T. cruzi* infection. The illiterate group had an OR=8.46, 95% C.I. = 1.00-392.11 relative to the group with more than 8 school years. The group with 1 to 8 school years had an OR of 3.44 and 95% C.I. of 0.52-145.71 when compared to the group with more than 8 school years. The Chi square for linear trend was statistically significant (p=0.016) in this case. Moreover, *T. cruzi* positive women were more likely to be older than 30 years (OR = 4.82, 95% C.I. = 1.60-17.37) and born outside Salvador (OR = 6.06, 95% C.I. = 1.79-31.89) than reference groups (Table 3). Other associations were not statistically significant.

Serological evidence for Syphilis was demonstrated by the presence of specific treponemal antibodies. A total of 40 (3.91%) women were positive (Table 1). The only variable associated with *T. pallidum* reactivity was spontaneous abortions (p<0.05, OR = 4.24, 95% C.I. = 1.48-14.86).

HBs Antigen (Hbs Ag) and corresponding antibodies, were present in 0.6% and 7.0% of the women, respectively. Significantly higher prevalence of HBV markers was found in women with previous history of blood transfusion (p=0.014).

TABLE 3

Factors associated with *T. cruzi* positivity among low income pregnant women in Salvador

Variables	No. of women		OR ^a	95% - CI ^b
	Pos./total	%		
Age ^c				
<21	5/284	1.76	1.00	-
21-30	5/564	0.89	0.50	0.11 - 2.19
>30	14/176	7.95	4.82	1.60 - 17.37
Place of Birth				
Salvador	3/467	0.64	1.00	-
Outside Salvador	21/557	3.77	6.06	1.79 - 31.89
Literacy ^d				
Illiterate	6/101	5.94	8.46	1.00 - 392.11
1-8 school years	15/600	2.50	3.44	0.52 - 145.71
>8 years	1/135	0.74	1.00	-

^aOdds Ratio, ^bConfidence Interval, ^cChi Square for linear trend - (p = 0.00032), ^dChi Square for linear trend - (p = 0.011627)

The seroprevalence for rubella virus antibodies was 77.4%. A trend for the association between rubella and age was observed (p=0.005, Table 4). No association between rubella antibodies and other variables could be found.

TABLE 4

Factors associated with Rubella positivity among low income pregnant women in Salvador

Variables	No. of women		OR ^a	95% - CI ^b
	Pos./total	%		
Age ^c				
<21	208/284	73.24	1.00	-
21-30	437/564	77.48	1.26	0.89 - 1.77
>30	149/176	84.66	2.02	1.21 - 3.42

^aOdds Ratio, ^bConfidence Interval, ^cChi Square for linear trend (p = 0.00515)

A total of 698 women were evaluated for the presence of *T. gondii* antibodies (IgG and IgM). A seroprevalence of 2.87% and 69.34% were observed for IgM and

TABLE 5

Factors associated with *T. gondii* positivity among low income pregnant women in Salvador

Variables	No. of women		OR ^a	95% - CI ^b
	Pos./total	%		
Place of birth for IgG				
Pos Women				
Salvador	250/323	77.40	2.09	1.47 - 2.96
Outside Salvador	233/375	62.13	1.00	-
Age for IgM Pos Women ^c				
<21	9/182	4.94	3.20	0.63 - 21.83
21-30	9/391	2.30	1.45	0.29 - 9.84
>30	2/125	1.60	1.00	-

^aOdds Ratio, ^bConfidence Interval, ^cChi Square for linear trend = 3.418 (p = 0.06450)

IgG, respectively (Table 1). Place of birth was a significant risk factor with an OR of 2.09 and 95% C.I. of 1.47-2.93 for the IgG positive women born in Salvador. Interestingly, we have observed a trend for the association between IgM positivity and age group. However, a Chi square for linear trend was not statistically significant (p=0.06, Table 5).

DISCUSSION

Our present study showed that HTLV-I/II prevalence was nine times higher than the one found for HIV-1 in pregnant women living in Salvador. In addition, the majority of HTLV-I/II positive samples were typed as HTLV-I. This prevalence is similar to that observed in Sub-Saharan African countries and Caribbean basin^{3,9,10,12,16,22,29,33,41}.

It has been amply demonstrated that HTLV-I infection and its related diseases are endemic in some regions of Brazil^{1,5,7,13,21,23,24,27,28,32,35,37}.

But the highest HTLV-I prevalence in Brazilian blood donors was recently detected in Salvador³⁷. This city was the most important harbor during the Atlantic African Slave trade in Brazil and sociodemographic characteristics are linked to this period². Therefore, in this city HTLV-I could have originated from Africa. However, there are evidences for an Asian origin of HTLV-I in other parts of Brazil. Indeed, high prevalence rate in Japanese immigrants and their descendants mainly in São Paulo State, Southeast Brazil, suggests an Asian origin of that virus. Further molecular epidemiological studies could clarify this question²³.

We could not find a clear HTLV-I transmission risk factor since the positive women did not report any blood transfusion nor intravenous drug use. An elusive point of heterosexual transmission in this population was the evidence that HTLV-I positive women were more likely to have had more than two lifetime sexual

partners than reference single partner group. In addition, recently, some evidence was presented suggesting the sexual transmission of HTLV-I/II in Salvador³⁵. However, partners of HTLV-I, positive women could not be tested for HTLV-I. On the other hand, there was no statistical significant association between the presence of HTLV-I antibodies and serological markers for other sexually transmitted diseases such as syphilis, hepatitis or HIV-1 infection. Therefore, HTLV-I/II heterosexual transmission in Salvador remains an issue to be further investigated.

Because of the low prevalence of HIV-1 in our target population, we believe that heterosexual transmission has not become an important route for HIV-1 spreading in Salvador confirming our previous study.¹⁷

The seroprevalence of *T. cruzi* was lower than that previously found⁴. Seroreactivity for *T. cruzi* was more frequent in women older than 30 years, illiterate and born in rural areas or other endemic small cities. These data suggest that *T. cruzi* transmission takes place in poor areas outside Salvador, where active insect transmission still occurs. Thus, actions should be taken to improve general conditions of the population, specially directed to control parasite vector transmission and housing in these regions.

The seroreactivity to *T. pallidum* was statistically associated with a history of spontaneous abortion. This association suggests that this pathogen is an abortion-inducing agent in Salvador, as already observed in other poor communities³⁶.

Concerning rubella virus, there was evidence that the prevalence has not changed significantly over the last 10 years²⁶. This prevalence increases with age but a significant number of women at child-bearing age are still susceptible to rubella virus. Thus, a rubella vaccination program should avoid potential outbreaks of rubella.

An increased seroprevalence for *T. gondii* was observed as we detected a higher positivity than that found six years earlier²⁵. A rather high prevalence of IgM for *T. gondii* was observed in women younger than 21 years, suggesting a possible high risk for transmission of congenital toxoplasmosis as it has been observed in other parts of the country^{8,42}.

Regarding HBs antigens, we observed lower rates than that one reported eight years ago in pregnant women in Salvador³⁸. Due to the low prevalence of HBs antigen, we concluded that perinatally-transmitted HBV infection should not be considered epidemiologically important in Salvador.

In general, our study showed some different rates of perinatally-transmitted infections when compared with those found in Sub-Saharan African countries. This was clearly evident in the cases of HIV-1 and HBV^{14,18,20,43}.

Indeed, we found prevalence value for HBV comparable to that one observed in low endemicity areas¹⁹. In these areas, the most common routes of transmission are sexual contact or intravenous drug users. However, in Salvador, history of blood transfusion should be considered.

Concerning HIV-1, the prevalence observed in our present study is similar to those found in the Northern America and Western Europe⁶.

Furthermore, there is indication that HIV-1 transmission is still occurring in Salvador, Bahia, among individuals with risk behaviors. In spite of this fact, heterosexual and perinatal transmissions are increasing in other parts of Brazil⁴⁰.

Ideally, all women should be screened for any perinatally transmitted pathogen during the prenatal care. Based on our data, this recommendation should be followed in Salvador. Unfortunately, due to limitations of prenatal care resources in public health services, we recommend as mandatory, screening test for Rubella virus, *T.pallidum*, *T.gondii* and HTLV-I. On the other hand, screening tests for *T.cruzi* hepatitis and HIV-1 are recommended whenever risk factors are present.

In areas with higher prevalence for these infections mandatory screening should be considered for pregnant women.

In addition, it is of paramount importance the establishment of a sentinel surveillance program for pregnant women in order to monitor the trends of these infections.

Implementation of these recommendations will certainly prevent the transmission of several perinatally-transmitted pathogens in Salvador.

RESUMO

Soroprevalência do HIV, HTLV-I/II e outros patógenos de transmissão perinatal em Salvador, Bahia.

A obtenção de dados epidemiológicos é de fundamental importância para o estabelecimento de políticas em Saúde Pública. No Brasil, essas informações são escassas, principalmente na região Nordeste. Para se obter alguns destes dados, avaliamos a soroprevalência de algumas infecções de transmissão perinatal, em cerca de 1024 gestantes de baixa renda, em Salvador, Bahia. Os resultados encontrados foram os seguintes: HIV-1 (0,10%), HTLV-I/II (0,88%), *T.cruzi* (2,34%), *T.pallidum* (3,91%), vírus da rubéola (77,44%), IgM e IgG para *T.gondii* (2,87% e 69,34%, respectivamente), e antígenos e anticorpos de superfície (HBs Ag e anti-HBs) do vírus da hepatite B (0,6% e 7,62%, respectivamente). A prevalência de HTLV-I/II foi nove vezes maior do que aquela observada para o HIV-1. Constatou-se um decréscimo na prevalência das infecções causadas pelos *T.cruzi* e o vírus da hepatite B, em relação a anos anteriores, enquanto na infecção pelo *T.gondii* houve um aumento. Em função dos dados encontrados recomendamos que em Salvador, testes de triagem para HTLV-I, IgM, para *T.gondii*, *T.pallidum* e o vírus da rubéola, sejam feitos como rotina prenatal, e que triagens para *T.cruzi*, hepatite B e HIV-1 sejam feitas quando estiverem presentes fatores de risco associados a estas

infecções. Entretanto, em áreas com altas taxas de prevalência para estas infecções, a triagem no prenatal deve ser considerada.

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